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KINETOSOME-CENTRIOLAR DNA: SIGNIFICANCE FOR ENDOSYMBIOSIS THEORY

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SUMMARY

The *"extreme version"* of the serial endosymbiosis theory (SET) postulates three types of integration of bacterial symbionts into what became the nucleocytoplasm of nucleated cells. The first, a symbiosis between motile bacteria and less motile archaeobacterial hosts is thought to have resulted in the origin of nucleocytoplasm capable of both internal movement and motility by undulipodia. That is, microtubule-based motility systems of eukaryotic cells (kinetosome-centrioles, undulipodia including cilia, mitotic spindles, etc.) are hypothesized to have evolved from symbiotic associations of spirochetes with *Thermoplasma*-like archaeobacteria (protonucleocytoplasm). The second, a symbiosis between aerobically respiring bacteria and the nucleocytoplasm, resulted in mitochondria of aerobic eukaryotes. The third association led to photosynthetic plastids from undigested cyanobacteria. Whereas the symbiotic origins of plastids and mitochondria are firmly established, the recent discovery by David LUCK and his colleagues of centriole-kinetosome DNA greatly enhances the likelihood that the last postulate concerning motility is valid. We can thus anticipate a rapid, definitive test of the *"extreme version"* of the SET.

Unknown to most Western scientists, the hypothesis of the origin of eukaryotic cell motility from symbiotic bacteria has a Russian antecedent: the concept of *symbiogenesis* or origin of evolutionary novelty via hereditary symbiosis was well developed by Russian biologists late in the last century and early in this one. Indeed, Boris M. KOZO-POLYANSKI, who wrote only in Russian, suggested that cilia derived from *"flagellated cytodes"*, by which he meant motile bacteria.

New results from molecular biology lend strong support to the SET, a theory which forms the foundation of the concept of symbiogenesis. Both the SET (which explains the genesis of the first eukaryotic cells) and symbiogenesis (a general evolutionary process involving heritable transmission of symbiotically acquired characteristics) require that all eukaryotic organisms be viewed, at the cellular level, as complex microbial communities.

Personal recollections

Impressed with the frequency of cases of nonmendelian heredity in a world of nuclear inheritance (e. g., photosynthesis mutants of plants and algae, "petites" in yeast, cortical inheritance in *Paramecium,* etc.), as a genetics student at the Universities of Wisconsin (1957-60) and California, Berkeley (1960-65), I (L.M.), explored the early literature for clues for the explanation of cytoplasmic heredity. That there were no "naked genes" in eukaryotic cells became clear, and evidence for the presence of bacterial genetic systems inside them became equally obvious. On the basis of literature reviews of cytoplasmic heredity and my consequent predictions of organellar DNA, I wrote the statement of the origin of nucleated ("mitosing") cells from bacterial symbiotic associations in 1965. It was first published by James DANIELLI, co-originator of the lipoprotein bilayer membrane theory, in the *Journal of Theoretical Biology* after approximately 15 rejections of the manuscript (MARGULIS, under the previous name of SAGAN, 1967). The expanded version of the theory of the origin of eukaryotic cells organelles (mitochondria, plastids, centrioles in nucleocytoplasm) was developed into a book originally under contract, and then rejected, by Academic Press. Eventually published by Yale University Press (MARGULIS, 1970), the theory itself was supported during the decades of the 70's and 80's by many new results derived from molecular biological, genetic and ultrastructural studies. The revised version of the work became the monograph *Symbiosis in Cell Evolution* (MARGULIS, 1981). Because of the importance of the discovery of kinetosome/centriole DNA described here, a second edition of the 1981 book is scheduled for publication in 1992.

The theory of the derivation of nucleated cells from a series of bacterial symbioses was named *Serial Endosymbiosis Theory* (SET) by TAYLOR (1974). F. J. R. (Max) TAYLOR, Canadian marine biologist and expert on dinomastigotes, attempted –with limited success– to develop the details of the contrasting nonsymbiotic origin of eukaryotes. As intellectual exercise he described the origin of organelles by "direct filiation" or autogenesis (TAYLOR, 1976). He also distinguished different versions of the views presented here: the xenogenous view of organelle origin, i.e., the serial endosymbiosis idea. He collected data concerning the possible origins of mitochondria (from respiring bacteria) and plastids (from cyanobacteria). The concept of the origin of plastids (but not mitochondria) or the origin of both plastids and mitochondria by symbiosis was labeled the "mild version" of the SET. TAYLOR called my theory the "extreme version" of the SET for I insisted that the kinetosomes, cilia and related microtubule organelles (Fig. 1) were also of symbiotic origin from motile bacteria.

As far as I knew at the time, mine was an entirely original contribution. In the 60's cell symbiosis ideas were to some extent still disreputable. Some scientists, like Hans RIS (who was my professor at the University of Wisconsin, Madison), knew well E.B. WILSON's (1928) masterpiece *The Cell in Development and Heredity,* and saw to it that articles written describing the discovery of DNA outside the cell nucleus in the early 1970's mentioned ideas of organellar origin by symbiosis. Although some authors did cite historical views of cell symbiosis, and noted ideas of the origin of mitochondria and plastids as presented by American (e. g.: WALLIN, 1927) and French authors (e. g.: PORTIER, 1918), these scientists writing in the 1960's and 1970's were extremely skeptical of hereditary endosymbiosis (Chapter 2 in MARGULIS, 1981). Indeed TAYLOR and I were conscious of both prejudice against

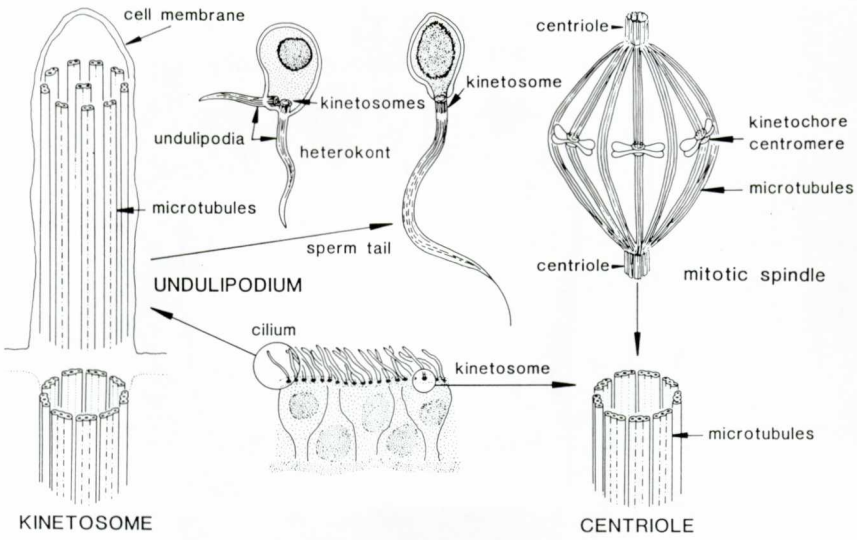

Figure 1. Microtubule-based organelles of eukaryotes from heterokont cells of algae and other protoctists, ciliated epithelium of animals, centrioles of animal and protoctist cells and mitotic spindles. If a shaft (axoneme) is present the basal structure is called a kinetosome, if absent it is a centriole.

and ignorance of cell symbiosis theories by "mainstream" experimental scientists. Yet, neither of us had knowledge of our Eastern European predecessors: the "symbiogeneticists" such as MERESCHKOVSKII and KOZO-POLYANSKI in Russia who, from the late 19th century until the 1950's (KOZO-POLYANSKI died in 1957) developed detailed proposals for the origin of evolutionary novelty, including cell organelles, by establishment of hereditary symbiosis.

Undulipodia before mitochondria

The original statement of the SET, on the basis of a preconception of monophyly of mitochondria, hypothesized the acquisition of these organelles prior to that of centriole/kinetosome/undulipodia. Because of the explosion of information on the eukaryotic microorganisms (protists and other protoctists, including so many lacking mitochondria altogether, MARGULIS *et al.*, 1990) and from ribosomal RNA sequence data (FOX, 1985), it has become clear that the acquisition of centriole/kinetosome/undulipodia preceded that of mitochondria and that mitochondria, derived from several types of respiring bacteria, are most likely polyphyletic within the protoctista (Fig. 2).

The recent spectacular discovery of kinetosomal-centriolar DNA, reported recently from the laboratory of David LUCK at Rockefeller University (HALL *et al.*, 1989) clarifies and refines the experimental evidence favoring the "extreme SET", and presents a severe challenge for competing hypotheses. Our purpose here is to place this work, achieved by genetic, cytological and molecular biological studies in the chlorophyte protist *Chlamydomonas,* in its appropriate historical context. The genetics of the motility system in *Chlamydomonas* needs to be better known; we hope to integrate these arcane genetic discoveries with the history of cell evolution concepts both in the USSR and the West.

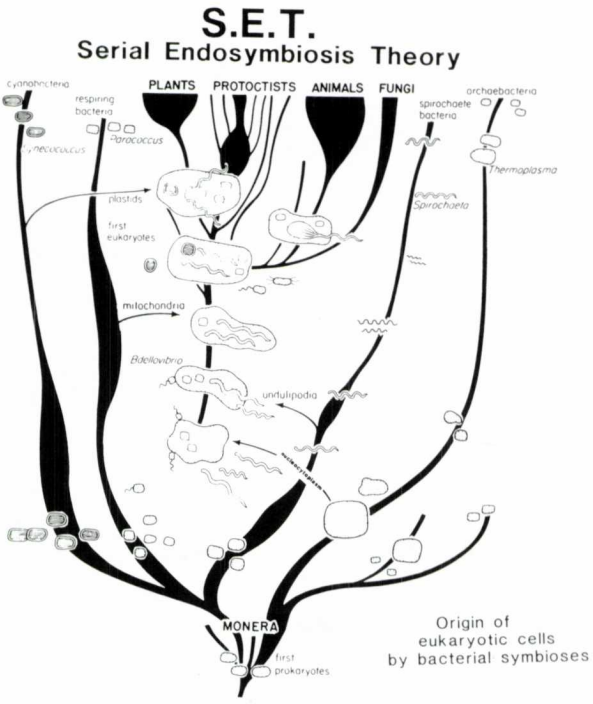

Figure 2. Serial endosymbiosis theory: diagram of the extreme version of eukaryotic cells origins with the order of organellar acquisition superimposed.

Kinetosomal DNA

Recently, the presence of a large quantity of DNA, 6-8 megabases, in the centriole/kinetosomes of *Chlamydomonas reinhardtii* has been reported (HALL *et al.*, 1989). Taking advantage of the fact that in *Chlamydomonas reinhardtii* mutants affecting kinetosomal assembly and undulipodia formation are clustered on a single linkage group, LUCK and his colleagues first showed that the position of these motility markers follows a circular pattern. Using pulsefield gel electrophoresis to resolve chromosome-sized DNA, they were able to identify the DNA corresponding the *uni* (also called "chromosone XIX") linkage group. Restriction fragment length polymorphisms detected with subclones of this DNA showed the expected 2:2 segregation patterns in crosses of *C. reinhardtii* bearing linked mutant markers with *Chlamydomonas smithii* wildtypes. *In situ* hybridization using cloned segments from two regions of the *uni* DNA as probes localized, with fluorescence microscopy, the *uni* DNA to the two kinetosomes. In *Chlamydomonas* undulipodial motility is lost during mitosis; these kinetosomes alter their morphology during the process of cell division and become the centrioles at the mitotic poles. By using known DNA standard chromosomes from yeast and *Neurospora,* HALL *et al.* (1989) estimate the size of the kinetosome/centriolar DNA to be very large, about 6 megabases.

Although the importance of this molecular analysis is evident, it is also worthy to note that geneticists and cytologists had anticipated this discovery. For some time, it has been observed that centrioles replicate in the cells (Fig. 3). They underlie and are required for the development of all undulipodia (i.e., cilia and eukaryotic "fla-

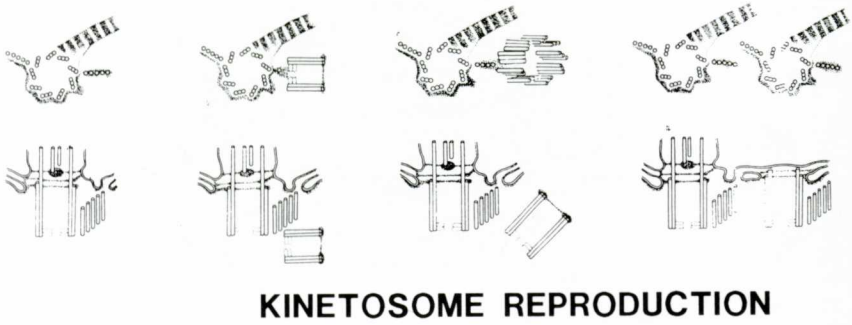

Figure 3. Kinetosome reproduction diagram based on electron micrographs of *Paramecium*. The [9(3)+0] transverse section is shown above and the longitudinal section below.

gella"). In many organisms, such as the protist *Lophomonas* (Fig. 4), "centrioles" and centromeres (= kinetosomes) can be seen to grow directly from undulipodial bases. When a shaft or axoneme is present they are called kinetosomes (or inadvisably basal bodies; MARGULIS and SAGAN, 1985), in the absence of an axoneme they are centrioles. Centrioles, as [9(3)+0] microtubular structures, often (i.e., in most animals), but not necessarily (i.e., never in plants), lie at each pole of the mitotic spindle. Centrioles have been called the "central enigma of cell biology" (WHEATLEY, 1985). Although often called flagella, all axoneme-bearing eukaryotic structures are entirely different from the flagella of bacteria. The term "flagellum" should therefore be avoided and *undulipodium* used. Figure 5 shows a comparative diagram of both the eukaryotic undulipodium and bacterial flagellum.

Especially in ciliate protists, kinetosomes and the pattern they make to form the cortex (outer 1 μm or so of the surface) are implicated in a weird inheritance system. In paramecia certain cortical mutants ("siamese-twin" or double-bodied cells) were induced using antisera and surgical needles by extremely skillful investigators (BEISSON and SONNEBORN, 1965). Doubled-bodiness was inherited through hundreds of generations. Genetic studies com-

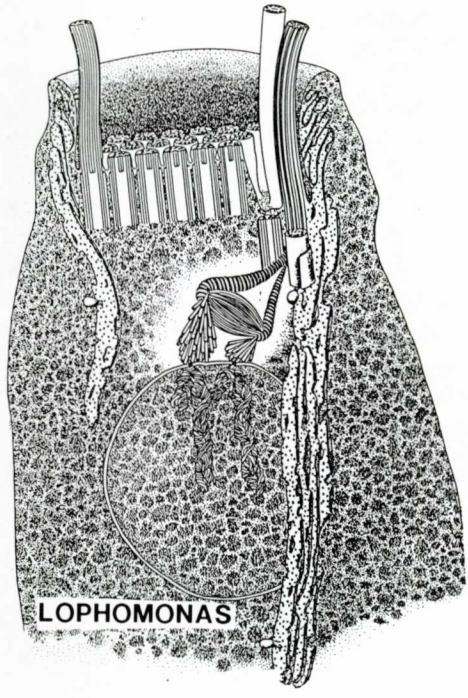

Figure 4. The connection between the kinetosomes and the mitotic spindle is clear in this drawing of the parabasalid symbiont of termites, *Lophomonas*. Based on electron micrographs of André Hollande, one can see the nuclear membrane-embedded kinetochores and extranuclear spindle attached to kinetosomes by striated fibers.

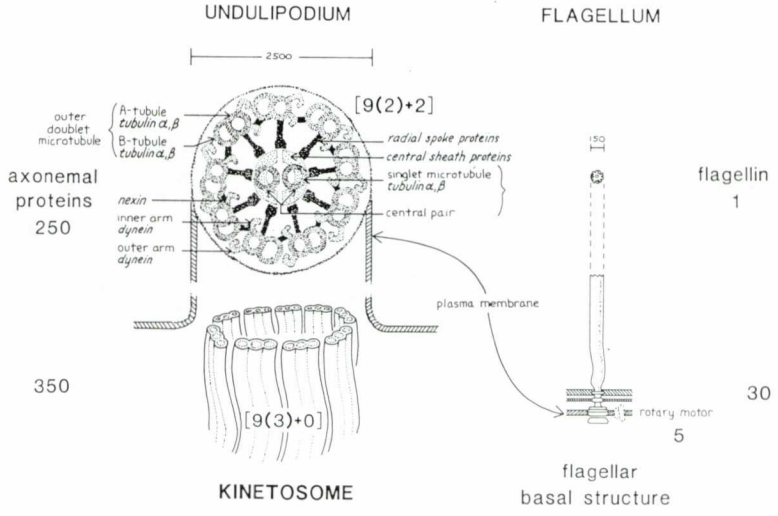

Figure 5. Comparison between the intracellular undulipodium and its kinetosomal base, that contain well over 500 proteins, with the much smaller and less complex extracellular bacterial flagellum, that contains fewer than 40 proteins.

pletely ruled out the possibility of nuclear gene control. Furthermore, because mitochondrial inheritance patterns of paramecia also can be manipulated by researchers, mitochondrial DNA control was excluded as the explanation for cytoplasmic inheritance of the doublet form. Other morphological mutants of paramecia ("swimmer", "snaky", etc.) also proved to be cytoplasmically –but not mitochondrially– inherited. Cortical inheritance is not under nuclear gene control. In spite of the fabulous quality of ciliate cortical genetics these remarkable cases of the inheritance of acquired characteristics are unappreciated in *vade mecum*-type, enormous genetics textbooks.

As discussed in *Microcosmos* (MARGULIS and SAGAN, 1986b), the discovery of kinetosomal DNA (kDNA) –or if not DNA, at least some remnant nucleic acid system– was anticipated. We are delighted that LUCK and his colleagues performed such elegant experiments. *Chlamydomonas,* the green protist with two kinetosomes per cell, well-marked nucleus, single chloroplast and controllable life cycle is the perfect haploid organism, very familiar to geneticists, in which to have sought kDNA. During each mitotic division kinetosomes resorb their axonemes and each naked kinetosome becomes a mitotic centriole in *Chlamydomonas,* therefore unlike in many other protoctists and animals, each centriole/kinetosome is capable of genetic continuity. We do not expect all mature kinetosomes to contain DNA; indeed those not capable of reproduction probably lack DNA at all times (YOUNGER *et al.*, 1972). The use of Luck's DNA probes, highlighted with a fluorescent label, allowed the visualization of the two tiny kinetosomes (each approximately 0.25 μm in diameter) per *Chlamydomonas* cell. They were highlighted on the cover of the journal *Cell* (HALL *et al.*, 1989). Such probes must now be used in other centriole-kinetosome containing organisms.

If this centriolar-kinetosomal DNA turns out to be the "spirochetal secret agents" predicted by MARGULIS and SAGAN (1986a), then all microtubule-making eukaryotes must have some DNA homologous to *Chlamydomonas* kDNA within

their cells even if, at certain times, the DNA takes up nuclear residence. If the intracellular motility system of all animal and plant cells evolved from symbiotic bacteria, such as members of the genus *Spirochaeta* (BERMUDES *et al.*, 1990), it is logical that a remnant bacterial DNA is detectable in all descendants of this event, even if it is 1500 million years after the symbioses were established (MARGULIS, 1981; BERMUDES *et al.*, 1987a). A strong case can be made that the evolution of the nucleated cell was a consequence of integration of motile spirochete symbionts which contained microtubules like that in Plate 1a. Such symbiotic spirochetes with lithe fast-swimming habits conferred rapid motility upon their sluggish partners. Spirochete-host genetic integration including membrane proliferation-initiated eukaryosis: evolutionary steps in the origin of mitosis and eventually meiotic sex (MARGULIS and SAGAN, 1986b). Epibiotic attached spirochetes and other bacteria are known today; in some cases these bacterial symbionts are difficult to distinguish from undulipodia (Plate 1b).

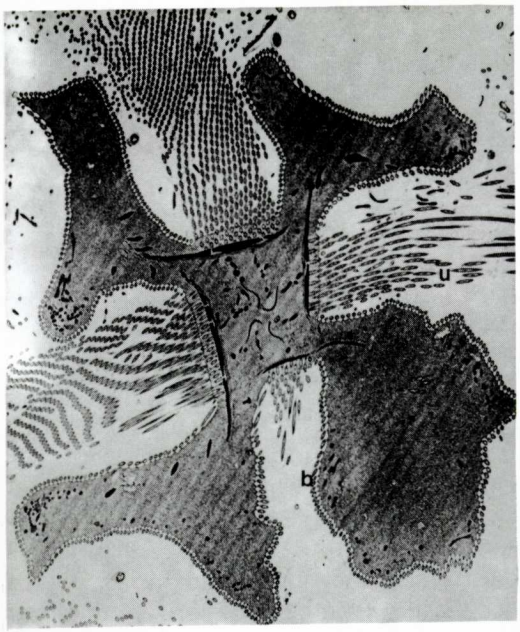

Plate 1a. Cytoplasmic tubules, approximately 24 nm in diameter, found by A. Hollande and I. Gharazoglou in the protoplasmic cylinder of *Pillotina* spirochetes (*mt:* microtubules; *m:* cytoplasmic membrane; *ri:* ribosomes; *n:* nuclear region).

Time of origin of centriole-kinetosomes

Frond-like organic films occur on bedding planes from the Belt Supergroup, Montana. These compressions are remains of algal thalli, possibly chlorophytes or phaeophytes (STROTHER, 1989). Thus 1300-1500 million years is a minimum age estimate for the origin of eukaryotes at the centriolar-kinetosomal grade of evolution, i.e., protoctista (MARGULIS *et al.*, 1990).

The Russian biologist Boris Michailovich KOZO-POLYANSKI (1890-1957) did not consider centriolar origin an enigma. To him, the centrioles –or their manifestations– could actually be visualized in living cells or iron-hematoxylin stained preparations: blepharoplasts and other cell centers were likely to be products of intracellular symbiosis (KOZO-POLYANSKI, 1924). They were intracellular structures, "organoids" [organelles], derived from "flagellated cytodes", by which he meant motile bacteria.

Symbiogenesis

Symbiogenesis, the evolution of novelty by integration of partners belonging to different taxa which remain in protracted physical association, had been a principle of evolution –at least in Russia– since the late 19th century. Symbiogenesis, a term coined by Konstantin Sergeivich MERESCHKOVSKII (1865-1921), explained the presence of greenish photosynthetic units in heterotrophs as diverse as hydra, diatoms and plant cells. MERESCHKOVSKII

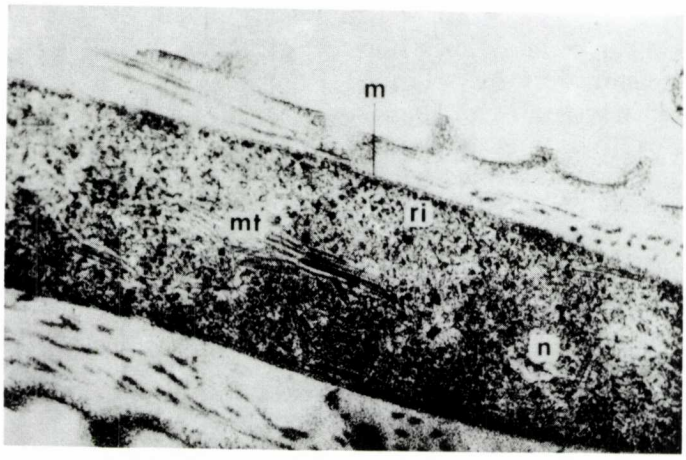

Plate 1b. The tendency to establish microbial symbionts is apparent in this photograph of *Staurojoenina*, a parabasalid protist containing both undulipodia *(u)* and unidentified surface rod-shaped symbiotic bacteria *(b)*.

(University of Kazan) and his theory of two plasms, together with his senior colleague from St. Petersburg, Andrei Sergeivich FAMINTZYN (1835-1916), was the most successful articulator of the theory of chloroplast origins as specific example of his general principle of symbiogenesis. These keen Russian biologists suggested –in contemporary terms– that plastids originated as captive cyanobacterial symbionts in heterotrophic cells. Although there was dialogue, mutual criticism and disagreement between these professors of biology, both down-played natural selection and both thought symbiosis to be crucial as an evolutionary mechanism (KHAKHINA, 1979). But it is KOZO-POLYANSKI who, in DYSON's terms, is our most "illustrious predecessor" in claiming the symbiotic origin of centrioles (DYSON, 1987). The position of KOZO-POLYANSKI on eukaryotic cell motility was clear:

Bodies known as blepharoplasts, immersed in the plasma of the cell and bearing a flagellum [undulipodium] or several flagella, come out from the cell interior. Blepharoplasts occur in mastigotes, flagellated cells of sponges and also spermatozoa; not only are they in animals, but, apparently, also in plants.

Many consider it demonstrated that blepharoplasts [kinetosomes] manifest themselves as a variety of centrosomes (or centrioles): the first may be transformed into the second and vice versa as seen microscopically in live cells. From the views stated here, the divisions of cells are synchronized... with the divisions of blepharoplasts (in the role of centrosomes or centrioles) –i.e., the motile flagella (or flagella-producing, or flagellated organelles or partners of the cell)... At least the suspicion of the bacterial nature of these kinetoplasmatic [motility] organelles without question appears entirely legitimate.

(KOZO-POLYANSKI, 1924; pp. 56-57).

Furthermore, in his book *New Concepts of Biology,* unlike his own illustrious predecessors MERESCHKOVSKII and FAMINTZYN, KOZO-POLYANSKI did not reject Charles Darwin. According to Liya Nicolaevna KHAKHINA, Soviet historian of science in Leningrad who researches history of symbiosis theories in evolution, KOZO-POLYANSKI recognized that natural selection plays a crucial part in symbiont

integration: if symbiosis is "author", natural selection is still "editor". Admitting natural selection, KOZO-POLYANSKI described the power of symbiogenesis using the term with the same original meaning of its inventor. In her book (1979) KHAKHINA cites MERESCHKOVSKII:

"I named this process symbiogenesis, which means: the origin of organisms by means of combination or conjunction of two or more beings, joined in symbiosis" *(Mereschkovskii, 1920). Symbiogenesis was considered by him as an evolutionary principle, which permitted a new approach toward resolution of the question of the origin of organisms. Working from his foundation, Mereschkovskii formulated evolutionary concepts which were called the "theory of symbiogenesis" (Mereschkovskii, 1909).* ***"These factual materials are based only on the accumulation of new facts from cytology, from biochemistry, and from physiology, principally from lower organisms, the aim of these attempts being to raise anew the veil obscuring secrets of the origin of organisms. I have resolved to make such attempts, and my work is the original... juxtaposition of previous statements of new theories of the origin of organisms, in which the phenomenon of symbiosis apparently plays a prominent role; I propose to call the theory symbiogenesis"*** *(Mereschkovskii, 1909).*

(KHAKHINA, 1979; p. 53)

But the language barrier was definitive: although works of both MERESCHKOVSKII and FAMINTZYN were cited by German and English speakers (WILSON, 1928), KOZO-POLYANSKI's contribution is virtually unknown outside the USSR. Fascinated, we note that the American anatomist (University of Colorado, Denver Medical School) Ivan E. WALLIN (1883-1969) developed *symbionticism,* an extremely similar theory of the role of symbiosis in speciation and cell organelle origin in the absence of interaction with the Russian symbiogeneticists. WALLIN was heartily disdained; his last article in 1965 was rejected by the journal *Science* (MEHOS, 1983). Although Paul PORTIER (1918, in France), Umberto PIERANTONI (1948, in Italy), and Paul BUCHNER (1965, in Germany) were all sympathetic to various degrees of symbiosis as a mechanism for the generation of evolutionary innovation, these authors were not fully "mainstream" scientists. The Russians, however, held important positions: MERESCHKOVSKII was professor at Kazan University, to Moscow the second most important university in Russia, and FAMINTZYN was director of the St. Petersburg (Leningrad) Plant Physiology Institute. Nevertheless, *symbiogenesis* was virtually unknown outside the Russian-speaking world where the concept, if not ignored, was labeled "controversial" or ridiculed (e.g.: LUMIÈRE, 1919).

For the serial endosymbiosis theory (SET) of cell origins to be definitively proven, Luck's centriolar DNA must be found generally in eukaryotes. Because it follows the distribution of microtubule organizing centers, this motility-associated DNA may be universal in meiotic organisms (e.g.: fungi, animals and plants), and nearly universal in protoctista, excepting those which lack microtubules, centrioles and kinetosomes (MARGULIS and SAGAN, 1986a; MARGULIS *et al.*, 1990). If DNA homologies are established between the appropriate spirochetal ancestors and these eukaryotes, then symbiogenesis must be considered anew. The inescapable inference is that animals, fungi and most protoctists had at least three microbial ancestors and all plants and algae had at least four. Evolutionary innovation for all eukaryotes involved far more than accumulation of mutations: it required integration of heterologous genomes. If all *animal cells* have at least three ancestors and all *plant cells* have at least four, how many heterologous ancestors has

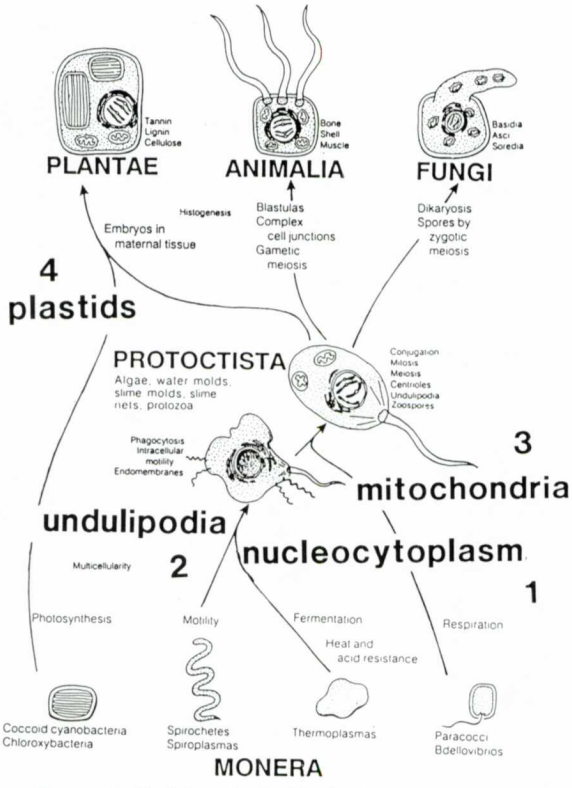

Figure 6. Serial endosymbiosis theory showing the origin of nucleated cells by bacterial symbioses: diagram emphasizing the polyphyly of the microbial ancestors.

a man, a cow or a weeping willow tree? Not only will the concept of "individual" be replaced with that of "symbiont" for all animals, but since all eukaryotes harbor heterologous DNA's from various sources both the sciences of eukaryotic evolution and of developmental biology transform: they become very special cases of applied microbial community ecology (Fig. 6).

Symbiogenesis as an evolutionary principle is under reconsideration (MAYNARD SMITH, 1989; LAW, 1989; MARGULIS and FESTER, 1991). Of course, that new organisms evolve by symbiont integration is not entirely new to the English speaking world: it is the explanation of choice for the origin of lichen structure, termite wood digestion, and the luminous organs in Leognathid fishes. But Luck's revelation of our spirochetal secret agents (if indeed kinetosomal-centriolar DNA's are present and homologous) uncovers the "anima" in all of us (MARGULIS and SAGAN, 1986a). No longer will the type of statement: *"all lichens are symbioses between algae and fungi"* be limited to lichens. Future cell biology texts must begin with a description of how all eukaryotic cells are derived from co-evolved communities of symbiotic bacteria. Future general biology textbooks, in addition to explaining why "protozoa" (as *"single-celled animals")* is no longer a valid taxon, must laud the enormous success of the phototrophic, oxygenic bacterial endosymbionts that color our planet green.

In the seminal discovery of kinetosomal/centriolar DNA by HALL *et al.*, we see the potential of the powerful techniques of molecular biology to ferret out evolutionary relationships between our bacterial ancestors. The strength of their contribution is the brilliant application of these techniques to fundamental biological problems extending back more than a century and across several language barriers.

Note added in proof. The term *undulipodia* has a long history of usage in the Russian literature, but the origins of the term are obscure and the scientist who originated it is unknown to us. The following passage, excerpted with condensation from L.N. SERAVIN (pp. 10-11, 1967, *Advanced systems of Protozoa. Structure, mechanochemistry and physiology.* USSR Academy of Sciences, Scientific Council on Problems of Cytology. "Science" Publishing House, Leningrad Division, Leningrad; terms in square brackets are ours) demonstrates how a Russian biologist in the 1960's understood the term *undulipodia* to refer both to cilia and to eukaryotic flagella:

Cilia and [eukaryotic] flagella are thread- or filament-like attachments to the surfaces

of protozoan [protoctist] cells. These organelles are capable of rhythmic motion, useful either for propelling the cell or for stiring the surrounding fluid.
*If these elongated objects are numerous on the cell surface and short, they are called cilia; if they are long and few in number they are called flagella. The activity of cilia of a single cell is coordinated, whereas flagella of a single cell function relatively independently. The distinctions, however, between these two types are not clear cut and there are a series of gradations between the two types. Electron microscope studies demonstrate that both organelles have a shared structure, and a single concept can be utilized to denote both types. A.P. Shmagina (1948) calls them undulipodia (*unda-*wave,* podos-*foot), the organelles of motion of the protozoans [protoctists].*
Undulipodia consist of two parts –the part outside of the cell (referred to as "proper" undulipodia) and the basal body (the part of the undulipodium within the cell). In some organisms fibrous structures attach to the basal body.
Undulipodia length varies greatly in different groups (ranging from 3 microns to 150 microns), but undulipodia length has an approximately constant value for all lengths. The distal section of undulipodia called achronemes is often observed to narrow distally, however.
(SMAGINA, A.P. 1948. *Ciliary Movement.* State Publishing House for Medical Literature "MEDGIZ", Moscow.)

Acknowledgements

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